also discuss ligand migration pathways and protein dynamics upon CO photo-release in rHb1.

289-Pos Board B69
The "Caged" State, the Transition State of the Regulation of Oxygen-Affinity in Hemoglobin
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The "caged" state of hemoglobin (Hb) and cobalt-substituted Hb (Co-Hb) is produced by cryogenic photolysis of respective hemoproteins at 4.2K [1-4]. The "caged" state of oxy-Hb and oxy-CoHb, in which the heme-oxygen bond is broken and the un-bonded oxygen is trapped in or near the heme pocket within the globin moiety, with spectral features distinct from those of either deoxy- or ligated states of respective hemoproteins, is the critical transition state in the regulation of the oxygen-affinity of Hb and CoHb. The oxygen-affinity of Hb is regulated by heterotropic effectors without detectable changes in either static quaternary/tertiary molecular structures of the protein or the coordination/electronic structures of the hemes and, thus, without changes in the oxygen-affinity of the heme Fe itself [5,6,8]. The reduction of the apparent oxygen-affinity of Hb may be caused by increase in the dissociation rate of oxygen from the "caged" state, resulting from effector-linked, enhanced high-frequency thermal fluctuations [6-8], which may simultaneously reduce the rate of geminate-recombination of oxygen to hemes. Thus, the oxygen-affinity of Hb is regulated by protein dynamics, rather than static structural changes. Thus, the "caged" state of Hb is the pivotal point in regulation of the affinity for small diatomic ligands such as oxygen, CO, and NO of Hb and Co-Hb [8].
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290-Pos Board B70
Understanding Thermodynamics of Conformational Change in the F-ATPase
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The F_0F_1-ATPase is a complex nanomotor that synthesizes nearly 90% of the ATP made during cellular respiration. It consists of two coupled rotary motors: an integral membrane complex driven by proton flow across lipid bilayers (F_0) and an enzymatic complex that converts ADP and inorganic phosphate to ATP (F_1). The rotational portion of these motors acts as a camshaft, inducing conformational changes that lead to ATP synthesis in the F_1 motor's three functional catalytic sites. The F_1 motor can perform ATP synthesis in the absence of F_0, and it can also work in reverse, hydrolyzing ATP to pump protons against an established gradient. Over the last 30 years many important aspects of this motor's function have been elucidated by careful biochemical work and further understood by clever biophysical experiments. However, there is still not a complete, quantitative description of the whole thermodynamic cycle—one that fully describes the interactions between all three separate catalytic sites and accounts for the need to exchange ATP (found abundantly) for the relatively sparse ADP. In the current work, we are using both molecular dynamics simulations and dynamic Monte Carlo methods to build a quantitative model of F_1-ATPase function.

291-Pos Board B71
Kinetic Control of O_2 Reactivity in H-Nox Domains
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Resonance Raman, transient absorption and vibrational coherence spectroscopies are used to investigate the mechanisms of NO and O_2 binding to *Tt*-HNOX and its P115A mutant. Vibrational Coherence spectra of the oxy-complexes provide a clear evidence for the activation of an iron-histidine mode around 217 cm^{-1} following photoexcitation, indicating that O_2 dissociates in both proteins. The quantum yield of O_2 photolysis is very low, particularly in the wild type. Geminate recombination of O_2 and NO in both proteins is very fast and highly efficient. This indicates that the distal heme pocket in these proteins is tightly packed, and forms an efficient trap, preventing the bound ligand from escaping into the solvent upon thermal dissociation. This, along with the the stabilization of the Fe-O_2 bond, explains the unusually high O_2 affinity in *Tt* H-NOX and its P115A mutant.

292-Pos Board B72
Probing Fleeting Interactions in Large and Dynamic Nonribosomal Peptide Synthetases with Novel NMR Methods
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Nonribosomal peptide synthetases (NRPSs) are modular, multi-domain, enzymatic assembly lines that combine simple substrates to synthesize complex natural products (e.g. penicillin, bacitracin, yersiniabactin). Substrates are each covalently attached to so-called carrier proteins (CP) via a post-translationally introduced phosphopantetheine arm. CPs present the substrates to catalytic domains in a series of transient, sequential domain/domain and domain/substrate interactions during biosynthesis. Thus, understanding the mechanism of NRPS assembly line synthesis necessitates both characterizing fleeting molecular interactions and unraveling the molecular determinants for the directionality of these interactions. Unfortunately, the large molecular weight of NRPS domains, the presence of dynamics between domains and within domains, and the lability of tethered substrates have precluded studying these processes at the molecular level. Here, we present methods that permitted us to overcome these limitations and we discuss our findings. First, by exploiting the non-invasive nature of NMR we found that chemical substrates interact with their carrier proteins and these interactions may modulate domain communication to provide directionality. Second, we present a method that facilitates resonance assignments in large proteins. By applying covariance to conventional spectra, simplified correlation maps permit to identify sequential residues or to assign side-chain resonances with a simple visual inspection. Hence, resonances of 37 kDa and 53 kDa NRPS domains could be assigned unambiguously, enabling us to describe molecular signatures of transient interactions involving these domains.

293-Pos Board B73
Visualizing the Inter-Domain Motions of a Pathogenic Protein using Sparse RDC Data
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SpA-N is the N-terminal half of Staphylococcal protein A and it is composed of five protein binding domains. The five domains could bind to antibody, TNFR1 and von Willebrand factor and facilitate the evasion of Staphylococcus aureus into the human immune system. The functional plasticity is suggested to be the result of structural flexibility. Heteronuclear spin relaxation experiments demonstrated that the five domains of SpA-N are connected by four flexible linkers. In order to obtain additional dynamic information, we constructed a di-domain mimic of SpA-N with a lanthanide binding tag and measured residual dipolar couplings (RDCs). The di-domain construct can bind lanthanide ions and consequently be aligned in the presence of a strong magnetic field. By using different combinations of lanthanide ions and protein constructs, we obtained two orthogonal alignments, which have enough information content to determine a model with maximally 10 parameters. In addition, we designed a de novo method to extract dynamic information from RDCs. Instead of determining conventional structure ensembles, our method determines an inter-domain orientation distribution to describe the structure of a flexible protein using continuous probability distributions. By using continuous models, orientation distributions can be parameterized by a small number of variables and still remain general enough to describe a broad spectrum of inter-domain motions. As a result, the method can avoid the over-fitting problem even with sparse data. Because no force field is applied, the generated distribution is purely determined by RDC data and least biased. Using the method, we determined the inter-domain orientation distribution of the di-domain construct with only two orthogonal alignments. A strong correlation was observed in the distribution and conformations were well populated in a limited region.

294-Pos Board B74
Mapping the Conformational Dynamics of the Scaffold Protein PSD-95
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Scaffold proteins form a dynamic framework to organize signal transduction by conjoining modular protein-binding domains. Scaffolds contain folded domains that are well understood but also disordered regions, which provide a